0022-202X/84/8203-0259$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 82:259–264, 1984
Copyright © 1984 by The Williams & Wilkins Co.
Vol. 82, No. 3
Printed in U.S.A.

Immunohistochemical Localization of Basement Membrane Components During Hair Follicle Morphogenesis

GILLIAN E. WESTGATE, B.SC., DENNIS A. SHAW, PH.D., GODFREY J. HARRAP, PH.D., AND JOHN R. COUCHMAN, PH.D.

Biosciences Division, Unilever Research, Colworth Laboratory, Sharnbrook, Bedford, U.K.

Specific antisera were used to investigate the distributions of several basement membrane zone (BMZ) components, namely, bullous pemphigoid antigen (BPA), heparan sulfate proteoglycan (HSPG), laminin, and type IV collagen, during the development of hair follicles in late embryo rats. BPA was not visible by indirect immunofluorescence in the BMZ before epidermal involution but appeared in all regions of BMZ after this had occurred. As follicular length increased during maturation, the distribution of BPA was no longer uniform, being reduced or absent from the BMZ around the lower part of the elongating follicle. HSPG was associated with the basal cell layer prior to the appearance of hair follicle primordia and became BMZ-associated before birth but after follicle buds were first observed. HSPG was also found to be associated with the basal cell surfaces in the epidermis, but not in the hair follicle. Laminin and type IV collagen were continually present in epidermal and follicular BMZ both before and during development of hair follicles and were later present in the dermal papilla matrix. From these observations we conclude that (1) laminin and type IV collagen are functionally important for BMZ integrity before and during epidermal and follicular maturation, (2) HSPG may have a cell surface function in epidermis as well as roles in BMZ organization and properties, and (3) the distribution of BPA is indicative of its association only with regions of tissue not involved in morphogenetic change. We also suggest that the cell–matrix interactions documented for BPA, HSPG, laminin, and fibronectin may depend on the type of tissue involved and its state of development, differentiation, or repair.

During skin development, inductive messages are transmitted from the dermis to the epidermis that lead to the ordered histogenesis of epidermis and determine the nature of the cutaneous appendages [1,2]. The mechanism of action of such molecular signals is poorly understood, but it has been proposed that the extracellular matrix at the dermal-epidermal junction plays an important role [3–5]. Components of this basement membrane zone (BMZ) in skin include the high-molecular-weight glycoproteins fibronectin [6,7] and laminin [8,9], type IV collagen [10,11], heparan sulfate proteoglycan (HSPG) [12,13], and the bullous pemphigoid antigen (BPA) [14,15](and see also review in [16]). The distribution of these components within the BMZ may vary from site to site [16,17] and although many interactions among them have been elucidated by in vitro analysis [18–20], the assembly and functional attributes of each component are far from understood. Whatever the organization of the BMZ, it must, however, provide a structural and functional base on which epithelia can function. Experiments have shown, for example, that BMZ components can influence the internal organization and behavior of epithelial cells as well as their proliferation [21].

The morphogenesis of hair in the rat is a rapid process occurring during the last few days of gestation. A fully stratified epidermis and functional hair follicles arise from 3 or 4 cell layers of undifferentiated epithelium, concomitant with dermal fibroblast specialization leading to dermal papilla formation. The later stages of skin embryogenesis therefore represent a dynamic system in which complex morphogenetic changes occur. In addition, a preformed BMZ has also been shown to be important for hair growth and development [22–24], and we have investigated the relative distributions of several BMZ components over this period.

We have previously studied the distribution of fibronectin in late embryo rat skin and have suggested possible roles for this matrix component during late embryogenesis and hair follicle development [7,25]. BPA has already been shown to be heterogeneously distributed in late embryonic and neonatal mouse skin [26], and we report here that BPA is absent from the lower follicular BMZ during the development of hair follicles in late embryo rat skin, but is present in the lower follicular BMZ during regressive and resting periods at the end of this and subsequent growth cycles. We show that laminin and type IV collagen are continually present in the BMZ during follicular development and appear in the dermal papilla matrix of more mature follicles. In addition, we have found that HSPG is present in the BMZ only after the first follicles begin to develop and we have observed a basal cell surface distribution of this proteoglycan in epidermis, hitherto not demonstrated.

MATERIALS AND METHODS

Preparation of IgG Fractions from Bullous Pemphigoid (BP) and Normal Human Sera

High-titer BP antiserum (kindly supplied by Dr. R. StC. Barnetson) or normal human serum from healthy adults was adsorbed, using 3-ml aliquots, onto a column of protein A-Sepharose CL4B, (0.7 g, final bed volume 2 ml, Pharmacia [Great Britain] Ltd., Hounslow, Middlesex). The column was washed with phosphate-buffered saline (PBS), pH 7.4, until no more unbound protein was eluted. The bound IgG fraction was then eluted with 0.58% acetic acid in 0.15 M NaCl and the eluate neutralized with solid Tris (2-amino-2-hydroxymethyl-propane-1,3 diol). This eluate (6 ml) was desalted and concentrated by ultrafiltration. The protein content of the final solution was 7 mg/ml and samples were freeze-dried and stored at −20°C.

HSPG Antiserum

Antiserum to Engelbreth-Holm-Swarm (EHS) tumor HSPG used in this study was kindly provided by Dr. J. R. Hassell and Dr. G. R.

Manuscript received July 19, 1983; accepted for publication October 27, 1983.

Reprint requests to: Dr. Dennis A. Shaw, Biosciences Division, Unilever Research, Colworth Laboratory, Sharnbrook, Bedford, U.K. MK44 1LQ.

Abbreviations:
BP: bullous pemphigoid
BPA: bullous pemphigoid antigen
BSA: bovine serum albumin
BMZ: basement membrane zone
EHS: Engelbreth-Holm-Swarm
FITC: fluorescein isothiocyanate
HSPG: heparan sulfate proteoglycan
IIF: indirect immunofluorescence
PBS: phosphate-buffered saline
Tris: 2-amino-2-hydroxymethyl-propane-1,3 diol

Martin [12]. The antiserum was adsorbed with laminin coupled to Sepharose CL4B (Pharmacia [Great Britain] Ltd.) before use. We have also used HSPG from liver cell surfaces and an antiserum raised against it, both of which were kindly provided by Dr. M. Höök [13,*].

Type IV Collagen Antiserum

Type IV collagen was extracted from human placentae by pepsin digestion [27] and precipitated from 0.5 M acetic acid with 1 M NaCl. Further purification was effected by repeated salt precipitation and ion-exchange chromatography [28]. Antibodies were raised in a New Zealand rabbit by subcutaneous injection of 1.2 mg of type IV collagen in complete Freund's adjuvant, followed by an identical injection 31 days later. The sera used were obtained from bleeds taken 43–108 days after the first injection. The specificity of the antiserum was demonstrated by inhibition of enzyme-linked immunosorbent assay [29]. The reaction was not inhibited by human placental type III collagen, type V collagen from human fetal membrane, or laminin from EHS tumor [8]. An immunoblotting technique [30] showed that the antiserum recognized all the major denatured fragments of prepared type IV collagen chains. Human, bovine, and rat collagen chains α_1(I) and α_2(I), human collagen chains α_1 (III), α_1(V), and α_2(V) did not bind the antiserum.

Laminin Antiserum

Rabbit antiserum to laminin derived from murine Reichert's membrane was kindly supplied by Dr. B. L. M. Hogan [31].

Tissue Preparation

Embryonic skin from 16- to 20-day-old house-bred albino Wistar CFHB rat fetuses was used, with the day of vaginal plug formation taken as day zero of embryonic growth. Skin from 16- to 18-day-old fetuses was carefully dissected and pieces stretched over segments of maternal liver before being snap-frozen for cryostat sectioning. Skin from 19- and 20-day-old fetuses was frozen flat in a double layer. Guinea pig esophagus or tongue (albino Duncan Hartley guinea pigs) was used for BP antibody titer measurements. Cryostat sections of 5–7 μm were made at −20°C and the frozen tissue sections either air-dried or fixed, according to the antibody used.

Indirect Immunofluorescent Staining (IIF)

Sections to be stained for BPA were air-dried and pretreated with 1% bovine serum albumin (BSA) for 15 min, followed by a 1:20 dilution of the reconstituted BP IgG fraction in PBS, and incubated for 30 min at room temperature in a wet-box. Following 3 washes with PBS, the sections were incubated for 30 min with a 1:32 dilution of sheep antihuman IgG conjugated with fluorescein isothiocyanate (FITC; F/P molar ratio 3.2, protein concentration 8 mg/ml, Seward Laboratory, London, England). The sections were rinsed thoroughly and then mounted in glycerol/PBS (9:1, v/v) containing *p*-phenylene diamine (0.1%), pH 8 [32]. Control sections were treated with normal human IgG fraction or PBS, followed by the FITC conjugate.

IIF for HSPG, laminin, and type IV collagen was carried out in a similar fashion. Sections were air-dried (HSPG only) or fixed for 10 min at room temperature with 3.5% freshly hydrolyzed paraformaldehyde in PBS, followed by washing in 0.1 M ammonium chloride in PBS. The anti-HSPG serum was used at a dilution of 1:5 in a 5% solution of BSA in PBS, antilaminin serum at a dilution of 1:50 in PBS, and the anti-type IV collagen serum at a dilution of 1:20 in PBS. FITC-conjugated goat antirabbit IgG (F/P molar ratio 3.5, protein concentration 10 mg/ml, Miles Laboratories, Slough, England) was used at a dilution of 1:40 in PBS. Sections were mounted in aqueous polyvinyl alcohol medium. Controls comprised: nonimmune serum, PBS (and others [31]), or 5% BSA in PBS, followed by FITC conjugate, for type IV collagen, laminin, and HSPG respectively. In the absence of a preimmune serum for HSPG, an antigen adsorption experiment was carried out using HSPG from liver cell plasma membranes [13,*] with the antiserum from the EHS tumor [12]. Sections of 20-day-old embryonic rat skin were used for IIF in this control.

All sections were examined on a Leitz Ortholux II microscope fitted with a Ploem vertical illuminator and fluorescence optics, and photographs taken on Ilford HP5, XP1, or FP4 films.

* Woods A, Höök M, Kjellén L, Rees DA, Smith CG: Relationship of heparan sulphate proteoglycans to the cytoskeleton and extracellular matrix of cultured fibroblasts. Manuscript in preparation.

RESULTS

Hair follicle primordia develop from the 16th day of gestation in fetal rat skin, the first indication being a subepidermal cluster of fibroblasts, which are cells of the presumptive dermal papilla (Fig 1*a*). From the 17th to the 20th day of gestation the follicular buds undergo rapid morphogenesis to produce an elongated follicle with a short hair cone and a fully developed dermal papilla (Fig 1*b*).

Bullous Pemphigoid Antigen

We found that BPA could not be detected by IIF in the BMZ at day 16 of gestation when hair follicle primordia were already present. It first appeared between days 17–18 as a faint discontinuous staining (Fig 2*a*), which became continuous between days 19–20 (Fig 2*b*). IIF staining for BPA remained continuous in the interfollicular BMZ during subsequent postnatal hair growth cycles. As the hair follicles enlarged in diameter and penetrated further into the dermis, the IIF staining for BPA in the follicular BMZ was diminished toward the lower end of the follicle (Fig 2*b*). This state persisted throughout the neonatal growing phase of the follicle when IIF staining for BPA was present only in the BMZ above the level of the sebaceous gland. However, IIF staining for BPA was observed in the lower follicular BMZ during follicle regression at the end of the first growth period (Fig 2*c*) and during the resting phase of the follicular growth cycle (Fig 2*d*). There was no IIF staining for BPA in the BMZ around the lower follicle during the growing phase of weanling and subsequent hair growth cycles; however, staining for BPA was observed in this region during the regressive and resting phases of these cycles. At no time during the development or growth of hair follicles was IIF staining for BPA observed in the dermal papilla matrix. Control sections at no time showed BMZ staining (not shown).

Heparan Sulfate Proteoglycan

At 16 days gestation HSPG was predominantly associated with the basal cell layer of the epidermis (Fig 3*a*), but whether it was localized to the BMZ at this time could not be discriminated. By 19 or 20 days, however, HSPG was apparently localized in the BMZ of both epidermis and hair follicles (Fig 3*b*) and basal epidermal cell surfaces were also stained (Fig 3*b*,

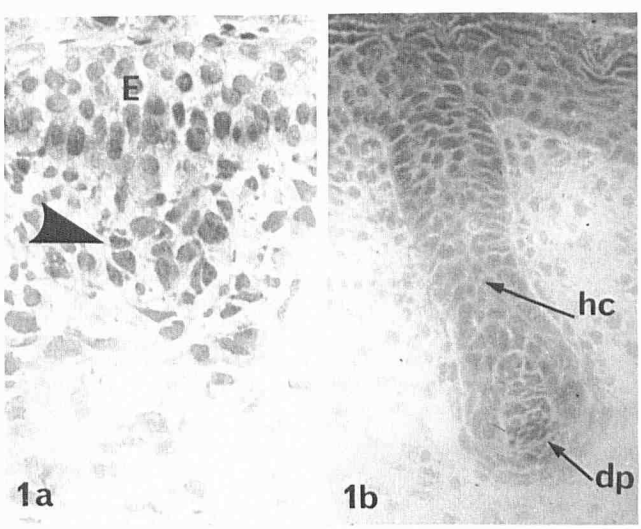

FIG 1. Rat skin from 16-day-old (*a*) and 20-day-old (*b*) embryos. The dermal fibroblast cluster shown in *a* (*arrowhead*) forms the dermal papilla of more mature follicles as shown in *b*. Hair formation originates with cells in the hair cone which is just discernable in 20-day-old embryonic skin shown in *b*. *E* = epidermis, *dp* = dermal papilla, *hc* = hair cone. Hematoxylin and eosin; *a* × 500, *b* × 200.

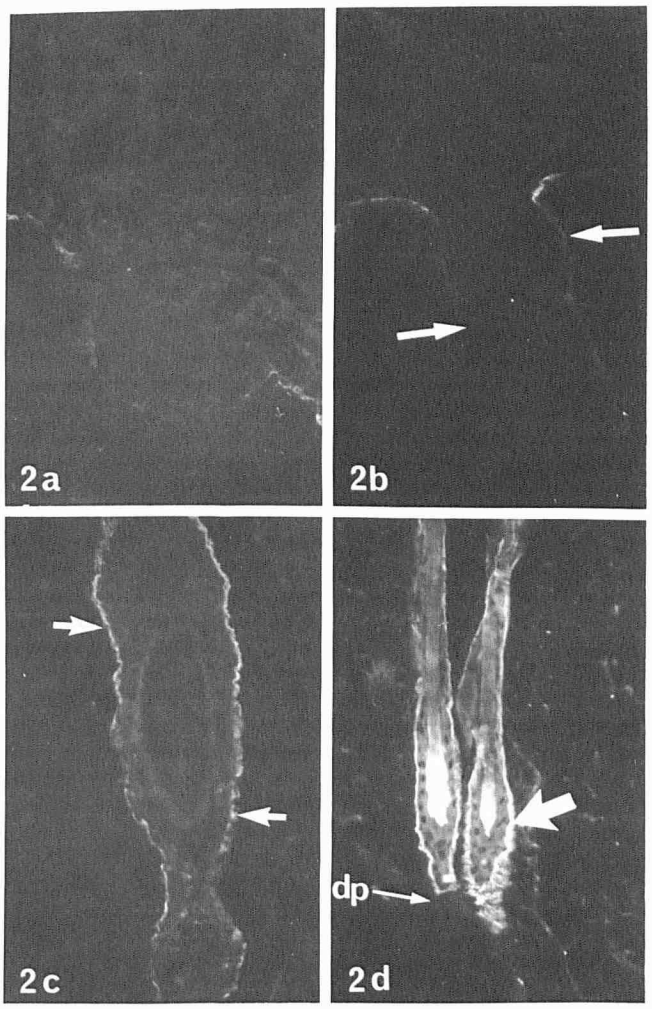

FIG 2. Indirect immunofluorescent staining of embryonic and postnatal rat skin using BP IgG. *a*, Sixteen-day-old embryonic skin where BMZ staining is faint and patchy. *b*, Twenty-day-old embryonic skin. Here staining is more intense and is continuous in the BMZ around the upper follicle and between follicles. However, there is no staining in the lower follicular BMZ. *c*, A follicle in 7-week-old rat skin which is in the regressive phase and demonstrates staining for BPA in the lower follicular BMZ. *d*, Two follicles in 3-week-old rat skin which are in the resting phase. From the follicle on the left of the figure it can be seen that there is no BPA associated with the dermal papilla. The staining in this region in the follicle on the right is due to the plane of section. *Arrows* indicate the follicular BMZ. *dp* = Dermal papilla. *a*, × 330, *b*, × 500, *c* × 200, and *d* × 270.

c). This cell-surface distribution of HSPG has not previously been demonstrated in vivo and did not extend to the follicular basal cells (Fig 3*c*). Controls using antiserum to EHS tumor HSPG that had been preadsorbed with HSPG from liver cell plasma membranes did not show this epidermal basal cell surface distribution of HSPG (Fig 3*d*). However, BMZ staining was sometimes observed, although it was faint and patchy (Fig 3*d*). This possibly indicates that the liver cell-derived HSPG did not bind all antibodies to HSPG, only those against cell-surface HSPG. Antiserum to liver cell-derived HSPG produces a staining pattern similar to that raised against EHS tumor HSPG, although BMZ staining is generally weaker (not shown). We consider therefore that this previously unobserved cell-surface distribution of HSPG in vivo is demonstrated by specific immunohistochemical staining. HSPG has been shown previously to be associated with cell surfaces in cell and organ culture studies (see discussion) and this is compatible with the results shown here.

Laminin and Type IV Collagen

Laminin and type IV collagen were present in the cutaneous BMZ at day 16 of gestation, both around involuting follicles (Fig 4*a*,*b*) and above areas of presumptive dermal papillae (not shown). These antigens were consistently present as BMZ components throughout the developmental period described here.

Staining was at all times continuous, no discontinuities being detected before or during the process of hair follicle formation (other than those attributable to sectioning artifact). However, the intensity of IIF staining was observed to increase toward birth. Follicles at day 19 or 20 of gestation showed the hair bulb enclosing the dermal papilla, and IIF staining for laminin and type IV collagen was observed in the BMZ around the dermal papilla and within the papilla matrix (Fig 4*c*,*d*). This "matrix" staining could have arisen partly from the basement membranes of capillaries that supply the dermal papilla. Stain-

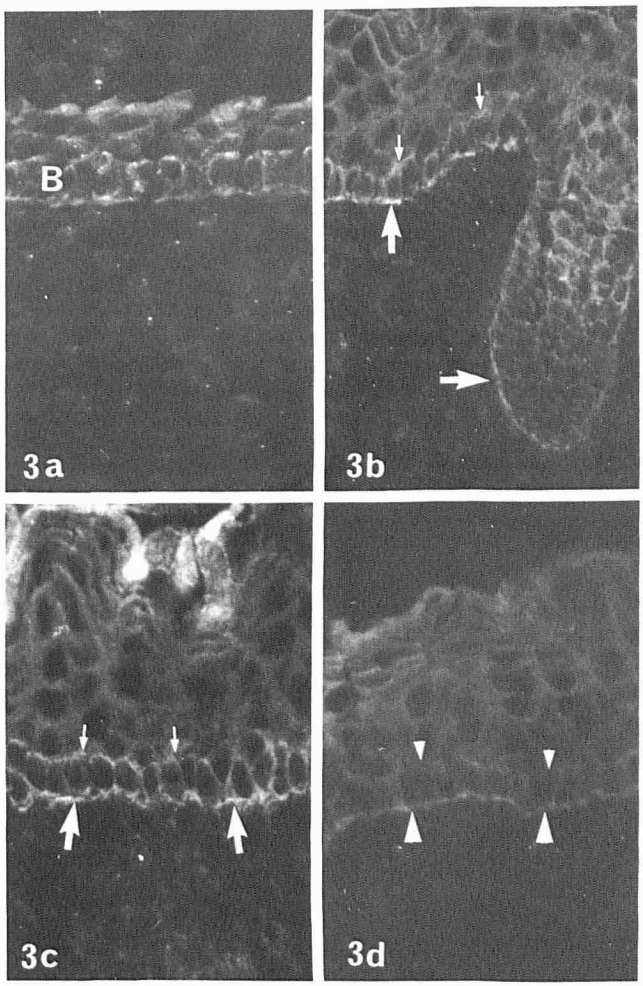

FIG 3. Indirect immunofluorescent staining of embryonic rat skin using antiserum to EHS tumor HSPG. *a*, Sixteen-day-old skin where staining is associated with the basal cell layer of the epidermis but does not appear to be in the BMZ. *b and c*, Twenty-day-old skin where staining for HSPG is associated both with the BMZ (*large arrows*) and with the basal cell surface (*small arrows*). *b*, Also shows BMZ staining around the hair follicle and demonstrates a lack of cell-surface staining in the basal layer of hair follicles when compared with that in interfollicular basal regions. *d*, "Control" shows 20-day-old embryonic skin stained with antiserum to EHS tumor HSPG that had been preadsorbed with HSPG from liver cell plasma membranes. There is noticeably less staining of the basal cell surfaces (*small arrowheads*) but some faint BMZ staining remained (*large arrowheads*). This demonstrates the specificity of the basal cell surface staining observed (*b* and *c*). *B* = basal cell layer. *a*, *b* and *d* × 330, *c* × 500.

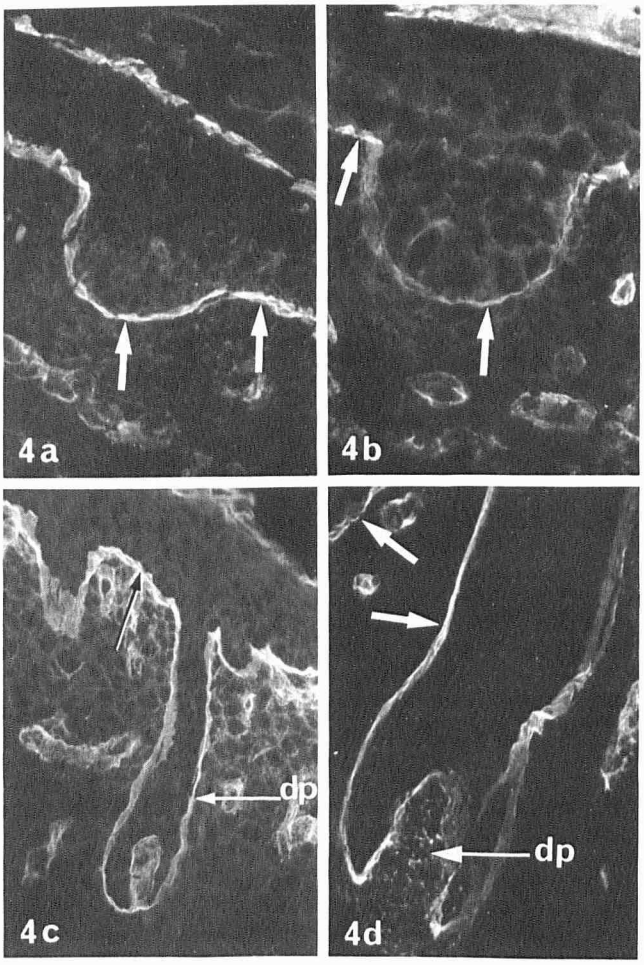

FIG 4. Indirect immunofluorescent staining of embryonic rat skin with antiserum to laminin (*a* and *c*) and type IV collagen (*b* and *d*). *a and b*, Sixteen-day-old skin where staining for laminin and type IV collagen is both intense and continuous in all regions of BMZ (*arrows*). *c and d*, Twenty-day-old skin where staining for these components is again intense and continuous both in follicular and interfollicular regions of BMZ (*arrows*). *c* and *d* also show staining for laminin (*c*) and type IV collagen (*d*) associated with the dermal papilla. *dp* = Dermal papilla. *a*, *b* and *d* × 500, *c* × 330.

ing of controls for laminin and type IV collagen was negative for all the basement membranes in skin.

DISCUSSION

This work was initiated to further the study of the relative distributions of known BMZ components in the later stages of embryonic skin development. The rapid maturation of the epidermis and development of hair follicles at this time are processes dependent on dermal-epidermal interaction [2,33], and our observations over this period have provided fresh evidence as to the possible roles of these BMZ components and the significance of their relative distributions in this dynamic system.

Bullous Pemphigoid Antigen

BPA distribution is known to be nonuniform in the BMZ of embryonic and neonatal mouse skin [22,26] and our results using rat skin support these findings. Such a heterogeneous distribution of a BMZ component highlights the possibility that basal cell-matrix interactions may be different for the epidermis and the lower hair follicle. Since BPA was observed at the dermal-epidermal junction (by IIF) and around follicle buds after their initial involution, it is likely that most basal epidermal cells are capable of synthesizing the antigen and depositing it into the cutaneous basement membrane. Indeed, in vitro studies have shown that basal epidermal cells synthesize BPA in culture [34,35]. Subsequent to epithelial specialization in areas of follicle bud formation, the newly forming basal cells in the growing follicle presumably either do not synthesize or do not deposit the antigen in the lower follicular BMZ, or its expression is prevented due to degradation or possibly antigenic masking. The appearance of the antigen during follicular regression could, by these same arguments, be due to either de novo synthesis by the basal cells abutting the follicular BMZ or an unmasking of the antigen in this region. Although masking and unmasking of antigenic determinants cannot be discounted as a possible cause of this phenomenon, it is perhaps likely that the presence or absence of BPA influences a particular type of cell–matrix interaction during the different phases of the follicular growth cycle.

It is possible, therefore, that in vivo BPA is involved with cell-matrix adhesion and evidence from cell culture studies supports this [34,35]. Such evidence, in conjunction with results from experiments using suction blisters [36] and pathologic bullae [15,37], is indicative of a role for BPA intimately involving the basal cell plasma membrane and its interaction with the underlying matrix. Recently it has been suggested that BPA may be necessary for cellular differentiation in the epidermis [38], and its close proximity to the basal cells may well reflect such a role for this BMZ component.

Heparan Sulfate Proteoglycan

Our results clearly demonstrate that HSPG distribution in skin is not restricted to the BMZ as was previously reported [12,39], but is specifically associated with the basal cell layer of the epidermis. The distribution seen here is consistent with other reports demonstrating a cell-surface distribution of this proteoglycan [40,*]. Cell surface-associated HSPG was not observed in the basal layer of the hair follicle where the stain was restricted to the region of the BMZ. Whether this was partly due to the plane of section could not be determined, but our results for BPA (above) have already indicated one difference between epidermal and follicular regions of BMZ. A heterogeneous distribution of basal cell-associated HSPG would also indicate that these two cell populations differ in functionality even though they are of common origin and are juxtaposed to a basement membrane. We have also observed that the antiserum to EHS tumor HSPG will stain both basement membranes and liver cell surfaces in the rat, which indicates that similar antigenic determinants are shared by HSPG from both locations (unpublished observations).

The role of HSPG in basement membranes is not known but it may function as a regulator of molecular traffic across the BMZ [41,42]. In this context it is significnt that we could not detect HSPG in the region of the BMZ of 16-day-old embryonic rat skin, i.e., at a time when molecular traffic across the dermal-epidermal junction may include signals for epidermal differentiation and follicular morphogenesis. It is not yet known how, or to what, HSPG is bound in basement membranes, although in vitro it is assembled into the extracellular matrix by both mesenchymal and epithelial cells [43,44] and may therefore mediate cell adhesion to collagenous matrices and their associated glycoproteins [45].

Laminin and Type IV Collagen

Laminin and type IV collagen are BMZ-associated in rat skin from day 16 of gestation and possibly earlier, and may be primary structural constituents of the BMZ. Their continued presence during follicle morphogenesis suggests that although there are extensive morphologic changes occurring that affect the relationship between the BMZ and basal cells of the elongating follicle, laminin and type IV collagen probably do not contribute directly to these changes but maintain BMZ integrity around the enlarging follicle. Some of the laminin and type

IV collagen observed in the matrix of the dermal papillae could originate from papillary blood vessels, but it is possible that these specialized fibroblastic cells synthesize a matrix for their own support which contains these matrix constituents. Fibronectin has been shown previously to be present in the dermal papilla matrices in embryonic and adult rat skin [7,25].

Although each of the BMZ components studied here has been implicated with roles in cell adhesion in vitro [46,18,45, 34], it may be that their precise functions in vivo are dependent on the state of development, growth, differentiation, repair, etc. of the tissues concerned. For example, BPA is associated with particular epithelial types [14], laminin synthesis coincides with BMZ formation and associated cell aggregation events [5,46], HSPG is thought to be important in tissue morphogenesis [47], and fibronectin may be important in the processes of cell migration and wound repair [48].

In conclusion, our studies have demonstrated that whereas some BMZ components are continually present in the BMZ both before and during late embryonic skin development and hair follicle morphogenesis (laminin and type IV collagen), others appear later and are subsequently heterogenously distributed as the epithelium gains new functionality (HSPG and BPA). In addition, we have highlighted that the embryogenesis of skin and hair is a suitable system for investigating the possible roles of basement membrane constituents in vivo at a time when morphogenetic activity in the skin is at a peak.

We thank both Mr. D. Illingworth for his contribution in establishing the immunofluorescence assay for BP antisera, and Mr. D. Chance for production of the photographic plates. We are indebted to Dr. R. StC. Barnetson (Royal Infirmary, Edinburgh) for providing us generously with BP antisera and to Dr. J. R. Hassell, Dr. G. R. Martin, and Dr. M. Höök for gifts of antisera to HSPG, and to Dr. B. L. M. Hogan for antiserum to laminin. Dr. I. Hughes-Hallett kindly collected normal human blood for us.

REFERENCES

1. Sengel P: Morphogenesis of Skin. Cambridge, England, Cambridge Univ Press, 1976
2. Dhouailly D: Dermo-epidermal interactions during morphogenesis of cutaneous appendages in amniotes. Front Matrix Biol 4:86–121, 1977
3. Vracko R: Basal lamina scaffold: anatomy and significance for maintenance of orderly tissue structure. Am J Pathol 77:313–346, 1974
4. Slavkin HC, Greulich RC: Extracellular Matrix Influences on Gene Expression. New York/San Francisco/London, Academic Press, 1975
5. Ekblom P, Alitalo K, Vaheri A, Timpl R, Saxén L: Induction of a basement membrane glycoprotein in embryonic kidney: possible role of laminin in morphogenesis. Proc Natl Acad Sci USA 77:485–489, 1980
6. Stenman S, Vaheri A: Distribution of a major connective tissue protein, fibronectin, in normal human tissues. J Exp Med 147:1054–1064, 1978
7. Couchman JR, Gibson WT, Thom D, Weaver AC, Rees DA, Parish WE: Fibronectin distribution in epithelial and associated tissues of the rat. Arch Dermatol Res 266:295–310, 1979
8. Timpl R, Rohde H, Robey PG, Rennard SI, Foidart JM, Martin GR: Laminin—a glycoprotein from basement membranes. J Biol Chem 254:9933–9937, 1979
9. Foidart JM, Bere EW, Yaar M, Rennard SI, Gullino M, Martin GR, Katz SI: Distribution and immunoelectron microscopic localisation of laminin, a non-collagenous basement membrane glycoprotein. Lab Invest 42:336–342, 1980
10. Timpl R, Martin GR, Bruckner P, Wick G, Weidemann H: Nature of the collagenous protein in a tumor basement membrane. Eur J Biochem 84:43–52, 1978
11. Yaoita H, Foidart JM, Katz SI: Localization of the collagenous component in skin basement membrane. J Invest Dermatol 70:191–193, 1978
12. Hassell JR, Robey PG, Barrach HJ, Wilczek J, Rennard SI, Martin GR: Isolation of a heparin sulfate-containing proteoglycan from basement membrane. Proc Natl Acad Sci USA 77:4494–4498, 1980
13. Kjellén L. Pettersson I, Höök M: Cell-surface heparan sulfate: an intercalated membrane proteoglycan. Proc Natl Acad Sci USA 78:5371–5375, 1981
14. Beutner EH, Jordan RE, Chorzelski TP: The immunopathology of pemphigus and bullous pemphigoid. J Invest Dermatol 51:63–80, 1968
15. Holubar K, Wolff K, Konrad K, Beutner EH: Ultrastructural localization of immunoglobulins in bullous pemphigoid skin. J Invest Dermatol 64:220–227, 1975
16. Briggaman RA: Biochemical composition of the epidermal-dermal junction and other basement membrane. J Invest Dermatol 78:1–6, 1982
17. Laurie GW, Leblond CP, Martin GR: Localization of type IV collagen, laminin, heparan sulfate proteoglycan and fibronectin to the basal lamina of basement membranes. J Cell Biol 95:340–344, 1982
18. Ruoslahti E, Pierschbacher M, Engvall E, Oldberg Å, Hayman EG: Molecular and biological interactions of fibronectin. J Invest Dermatol 79:65s–68s, 1982
19. Woodley D, Kleinman HK, Martin GR, Rao CN, Liotta LA: Interaction of purified basement membrane components (abstr). J Invest Dermatol 78:345, 1982
20. Stamatoglou SC, Keller JM: Interactions of cellular glycosaminoglycans with plasma fibronectin and collagen. Biochim Biophys Acta 719:90–97, 1982
21. Sugrue SP, Hay ED: Binding of fluoresceinated collagen and laminin to the corneal epithelial basal cell surface and the possible role of heparan sulfate proteoglycan (HSPG). J Cell Biol 95:136A, 1982
22. Leung Tack J, Thivolet J, Beyvin AJ, Schmitt D: Corrélations immunologiques et ultrastructurales au cours de l'ontogenèse de la membrane basale dermo-épidermique chez la souris. Annales de Dermatologie et de Syphilligraphie 98:525–528, 1971
23. Fraser DA: The development of the skin of the back of the albino rat until the eruption of the first hairs. Anat Rec 38:203–223, 1978
24. Briggaman RA: Basement membrane formation and origin with special reference to skin. Front Matrix Biol 9:142–154, 1981
25. Gibson WT, Couchman JR, Weaver AC: Fibronectin distribution during the development of fetal rat skin. J Invest Dermatol 81:480–485, 1983
26. Bard S, Micouin C, Thiovolet J, Sengel P: Heterogeneous distribution of bullous pemphigoid antigen during hair development in the mouse. Arch Anat Microsc Exp 70:141–148, 1981
27. Sage H, Woodbury RG, Bornstein P: Structural studies on human type IV collagen. J Biol Chem 254:9893–9900, 1979
28. Glanville RW, Rauter A, Fietzek PP: Isolation and characterisation of a native placental basement membrane collagen and its component α-chains. Eur J Biochem 95:383–389, 1979
29. Rennard SI, Berg R, Martin GR, Foidart JM, Robey PG: Enzyme-linked immunoassay (ELISA) for connective tissue components. Anal Biochem 104:205–214, 1980
30. Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76:4350–4354, 1979
31. Cooper AR, Kurkinen M, Taylor A, Hogan BLM: Studies on the biosynthesis of laminin by murine parietal endoderm cells. Eur J Biochem 119:189–197, 1981
32. Johnson DG, Nogueira Araujo GMdeC: A simple method of reducing the fading of immunofluorescence during microscopy. J Immunol Meth 43:349–350, 1981
33. Kollar EJ: The induction of hair follicles by embryonic dermal papillae. J Invest Dermatol 55:374–378, 1970
34. Stanley JR, Foidart JM, Murray JC, Martin GR, Katz SI: The epidermal cell which selectively adheres to a collagen substrate is the basal cell. J Invest Dermatol 74:54–58, 1980
35. Diaz LA, Marcelo CL: Pemphigoid and pemphigus antigens in cultured epidermal cells. Br J Dermatol 98:631–637, 1978
36. Woodley D, Sauder D, Talley MJ, Silver M, Qwarnstrom E: Localization of basement membrane components after dermo-epidermal separation (abstr). J Invest Dermatol 78:342–343, 1982
37. Hintner H, Stingl G, Schuler G, Fritsch P, Stanley J, Katz S, Wolff K: Immunofluorescence mapping of antigenic determinants within the dermo-epidermal junction in mechanobullous disease. J Invest Dermatol 76:113–118, 1981
38. Stanley JR, Beckwith JB, Fuller RP, Katz SI: A specific antigenic defect of the basement membrane is found in basal cell carcinoma but not in other epidermal tumors. Cancer 50:1486–1490, 1982
39. Thesleff I, Barrach HJ, Foidart JM, Vaheri A, Pratt RM, Martin GR: Changes in the distribution of type IV collagen, laminin, proteoglycan and fibronectin during mouse tooth development. Dev Biol 81:182–192, 1981
40. King IA: Characterisation of epidermal glycosaminoglycans synthesised in organ culture. Biochim Biophys Acta 674:87–95, 1981
41. Kanwar YS, Linker A, Farquhar MG: Increased permeability of the glomerular basement membrane to ferritin after removal of glycosaminoglycans (heparan sulfate) by enzyme digestion. J Cell Biol 86:688–693, 1980
42. Rosenzweig LJ, Kanwar YS: Removal of sulfated (heparan sulfate) or non-sulfated (hyaluronic acid) glycosaminoglycans results in increased permeability of the glomerular basement membrane to ^{125}I-bovine serum albumin. Lab Invest 47:177–184, 1982
43. Hedman K, Kurkinen M, Alitalo K, Vaheri A, Johansson S, Höök

M: Isolation of the pericellular matrix of human fibroblast cultures. J Cell Biol 81:83–91, 1979
44. Brown KW, Parkinson EK: Glycoproteins and glycosaminoglycans of cultured normal human epidermal keratinocytes. J Cell Sci 61:325–338, 1983
45. Kleinman HK, Klebe RJ, Martin GR: Role of collagenous matrices in the adhesion and growth of cells. J Cell Biol 88:473–485, 1981
46. Carlsson R, Engvall E, Freeman A, Ruoslahti E: Laminin and fibronectin in cell adhesion: enhanced adhesion of cells from regenerating liver to laminin. Proc Natl Acad Sci USA 78:2403–2406, 1981
47. Bernfield M, Banerjee SD: The turnover of basal lamina glycosaminoglycan correlates with epithelial morphogenesis. Dev Biol 90:291–305, 1982
48. Clark RAF, Lanigan JM, Della Pelle P, Manseau E, Dvorak HF, Colvin RB: Fibronectin and fibrin provide a provisional matrix for epidermal cell migration during wound reepithelialization. J Invest Dermatol 79:264–269, 1982